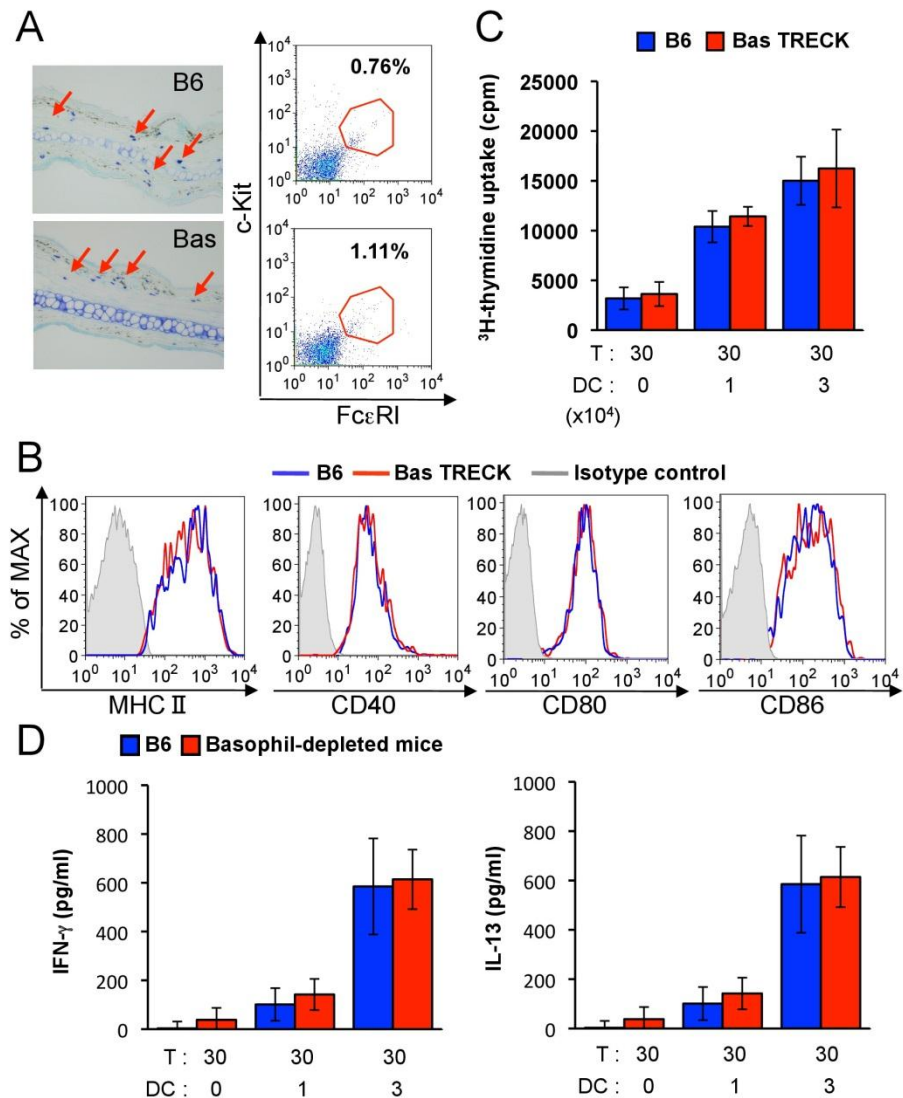


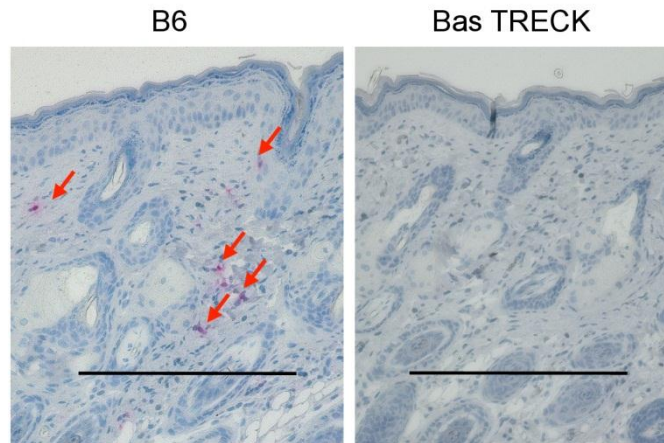
Supplementary Figure S1



Supplementary Figure S1. Mast cells and DCs are normal in Bas TRECK mice

(A) Skin mast cells in DT-treated B6 mice and DT-treated Bas TRECK mice were stained with toluidine blue (left panels). The numbers of mast cells in dermis of B6 and Bas TRECK mice was 11.8 ± 1.3 and 10.2 ± 2.1 per section, respectively. Flow cytometric data of skin mast cells (right panels). (B) The expression levels of MHC class II, CD40, CD80, and CD86 on CD11c⁺ DCs. (C) BALB/c CD4⁺ T cells were stimulated with DCs sorted from DT-treated Bas TRECK and DT-treated B6 mice in a mixed leukocyte reaction assay. (D) The levels of IFN-γ and IL-13 in the culture supernatant. (n = 4 per group). Data are presented as the mean ± SD and representative of three independent experiments with similar results.

Supplementary Figure S2

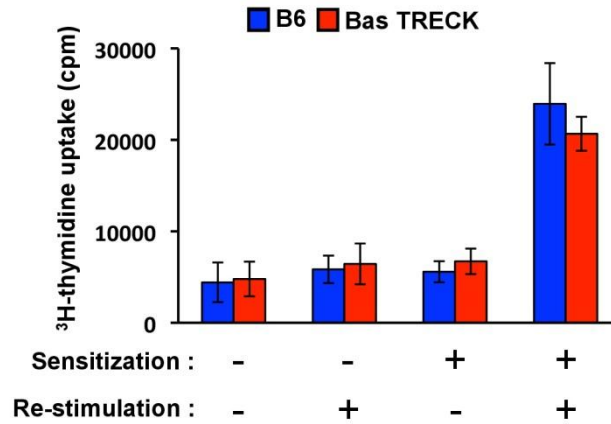


Supplementary Figure S2. Basophils accumulation in skin lesions in an OVA-induced allergic skin dermatitis model

Basophils were stained with anti-MCP8 antibody (TUG8) in the skin lesions of DT-treated B6 and DT-treated Bas

TRECK mice in an OVA-induced allergic skin dermatitis model (scale bar, 100 μ m).

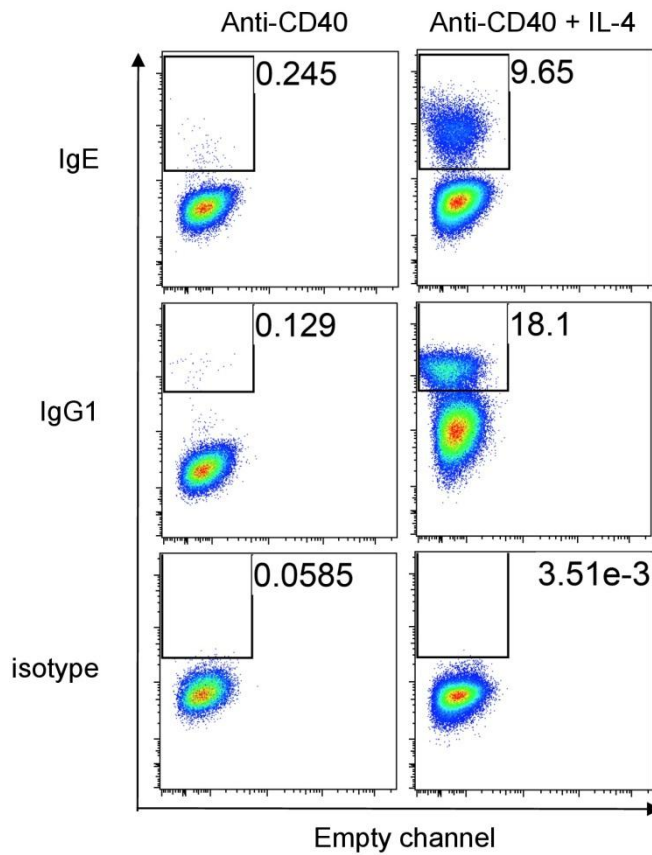
Supplementary Figure S3



Supplementary Figure S3. Basophils are not essential for T cell stimulation upon OVA protein exposure

DT-treated B6 and DT-treated Bas TRECK mice were immunized with OVA protein via a cutaneous patch. The skin-draining LN cells from B6 and basophil-depleted mice were re-challenged in the presence or absence of OVA protein *in vitro*. The incorporation of ^3H -thymidine in the presence of OVA was comparable between basophil-depleted mice and B6 mice. Data are presented as the mean \pm SD and representative of three independent experiments with similar results.

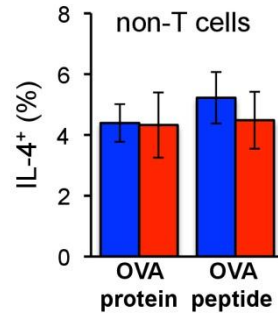
Supplementary Figure S4



Supplementary Figure S4. IgE and IgG1 induction model *in vitro*

B220⁺ B cells were sorted from splenocyte in B6 mice. 0.5×10^6 cells of B220⁺ were incubated with anti-CD40 (1 μ g/ml) and IL-4 (50 ng/ml) for 4 days. Cells were intracellularly stained with anti-mouse IgE, IgG1, or isotype control antibody and analyzed CD19⁺ B220⁺ cells

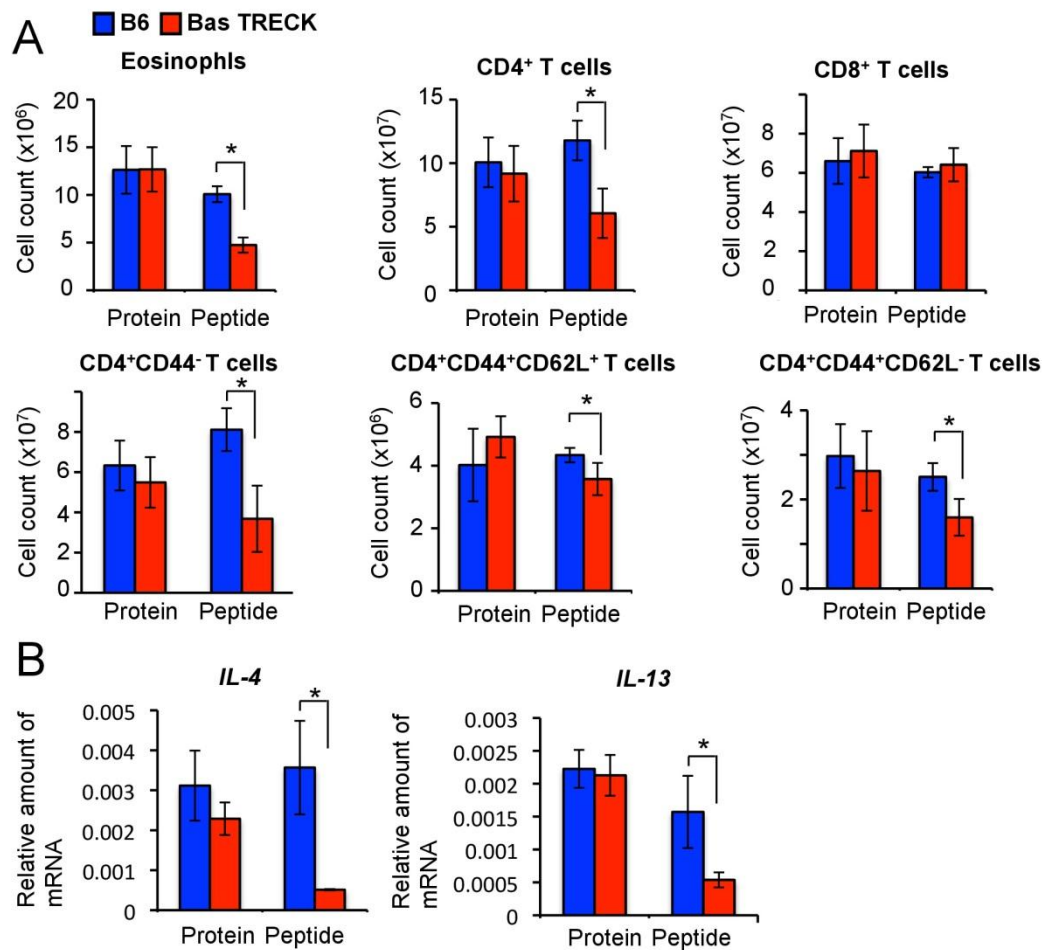
Supplementary Figure S5



Supplementary Figure S5. Intracellular cytokine staining for IL-4 in non-T cells upon intraperitoneal injection with OVA protein or peptide

The frequency of IL-4⁺ cell in non-T cells. (n = 4 per group). Data are presented as the mean \pm SD and representative of three independent experiments with similar results.

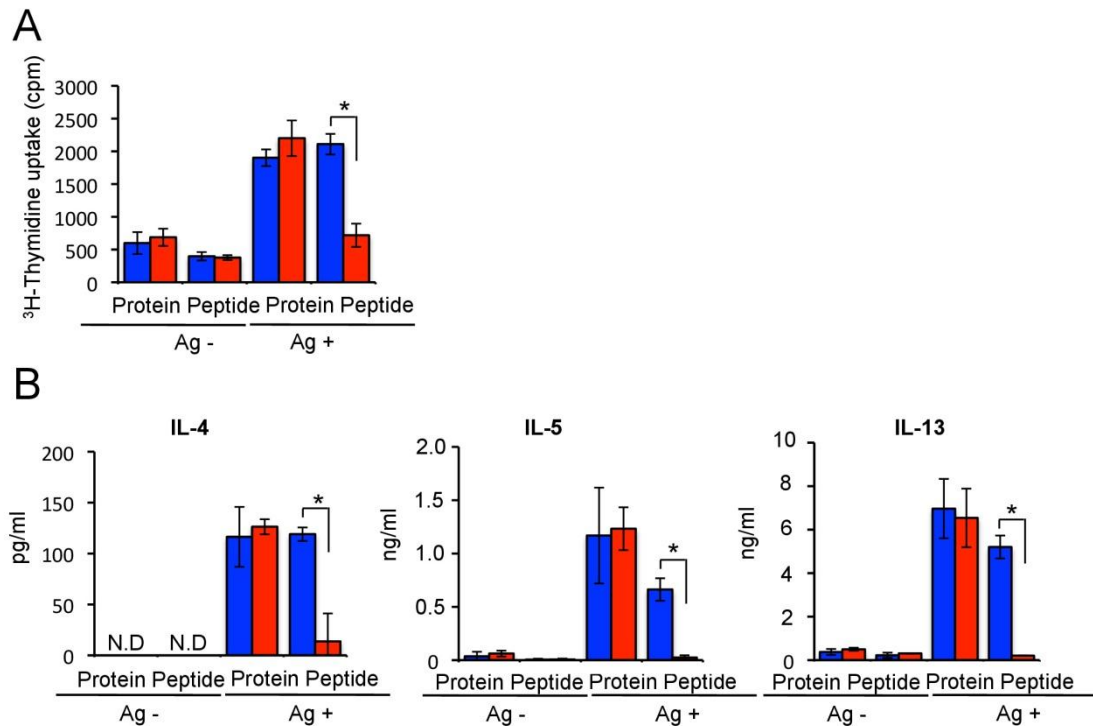
Supplementary Figure S6



Supplementary Figure S6. Impaired immune cell accumulation in DT-treated Bas TRECK mice upon peptide stimulation

(A) The numbers of eosinophils, CD4⁺, CD8⁺, CD4⁺CD44⁻, CD4⁺CD44⁺CD62L⁺, and CD4⁺CD44⁺CD62L⁻ T cells in spleen, and (B) the mRNA levels of *IL-4* and *IL-13* in the mesenteric lymph nodes upon intraperitoneal injection with OVA protein or peptide mixed with alum. (n = 4 per group). Data are presented as the mean ± SD and representative of three independent experiments with similar results.

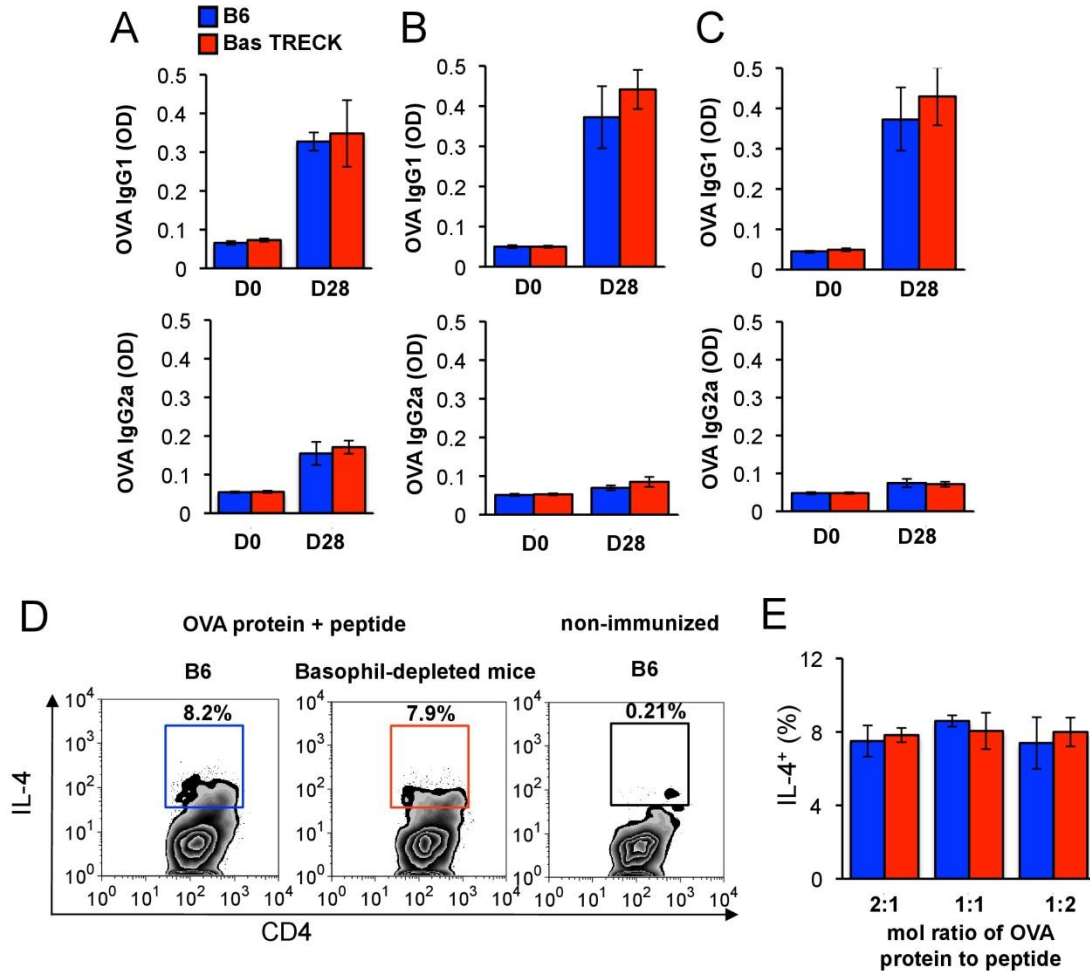
Supplementary Figure S7



Supplementary Figure S7. Attenuated T cell proliferation and Th2 response in DT-treated Bas TRECK mice to peptide antigen stimulation *in vitro*

(A) Splenocytes from DT-treated B6 and DT-treated Bas TRECK mice were challenged in the presence or absence of each antigen *in vitro*. The incorporation of ^3H -thymidine (A) and the levels of IL-4, IL-5, and IL-13 (B) in the culture supernatant ($n = 4$ per group). Data are presented as the mean \pm SD and representative of three independent experiments with similar results.

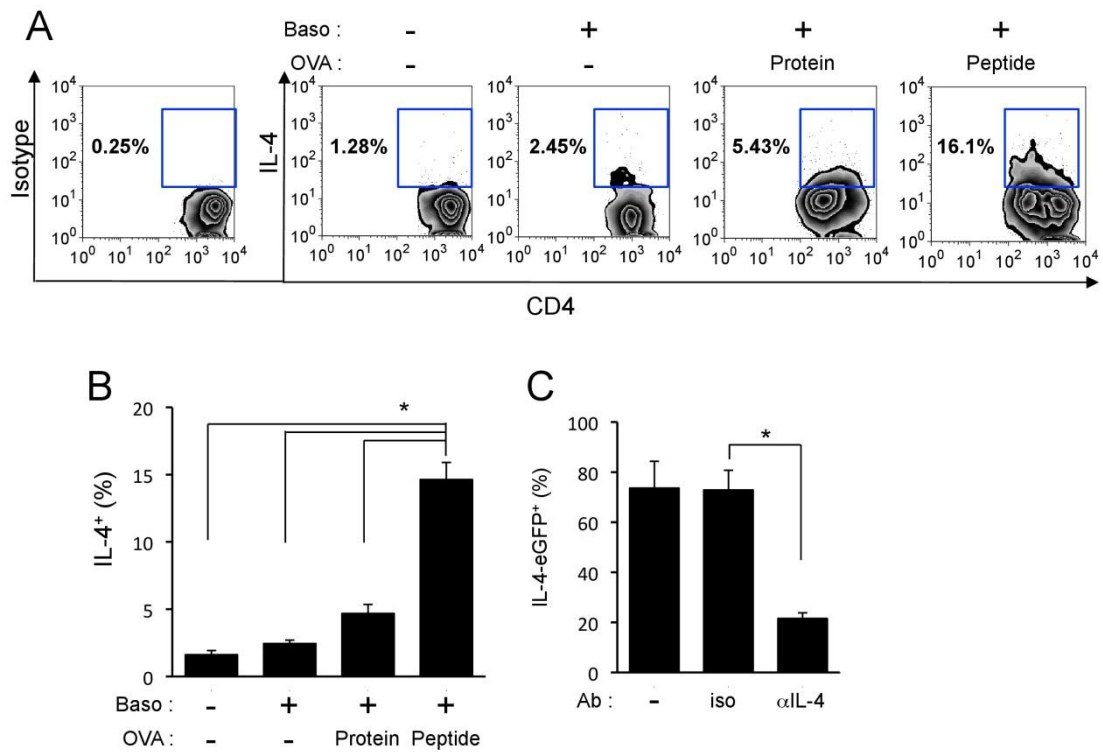
Supplementary Figure S8



Supplementary Figure S8. Comparable Th2 induction to both OVA protein and peptide in DT-treated Bas TRECK mice

To clarify whether basophils are necessary for Th2 cytokine responses in the context of complex inflammatory environments where presumably both small soluble antigens and larger proteins are immunogenic, both OVA protein and OVA peptide are used at the same time with alum. The levels of serum OVA-specific IgG1 and IgG2a on day 0 and 28 in different mol ratio (protein : peptide = 2:1 (A), 1:1 (B), and 1:2 (C)). (D) CD4⁺ T cells in spleen with intracellular cytokine staining for IL-4 (n = 4 per group) and the frequency of IL-4⁺ cells (E) in CD4⁺ T cells using these stimulated splenocytes. Data are presented as the mean \pm SD and representative of two or three independent experiments with similar results.

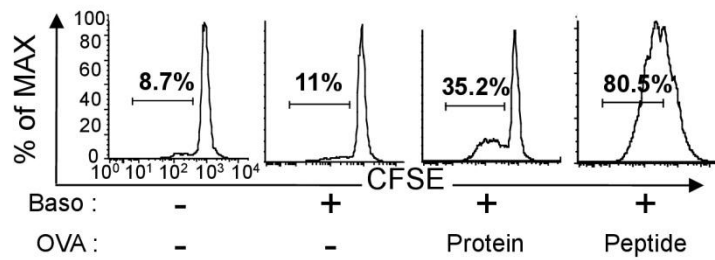
Supplementary Figure S9



Supplementary Figure S9. IL-4 protein induction by basophils in the presence of peptide

(A) CD4⁺ T cells were analyzed with intracellular cytokine staining for IL-4 after incubation in the presence of BM-derived basophils with or without OVA peptide or OVA proteins. (B) The frequency (%) of IL-4⁺ cells in CD4⁺ T cells after incubation. (C) The frequency of IL-4-eGFP⁺ cells on 4get T cells after incubation (as in Fig. 5G) with or without neutralizing anti-IL-4 antibody (iso: isotype control, αIL-4: anti-IL-4 antibody 50μg/ml) (n = 4 per group). Data are presented as the mean ± SD and representative of three independent experiments with similar results.

Supplementary Figure S10



Supplementary Figure S10. CFSE experiment after 3 day incubation

The populations of CFSE-diluted OVA-specific CD4⁺ T cells after 3 day incubation, as an indication for T cell proliferation, were shown.

	B6	Bas Tg
Inflammation	3.4 ± 0.6	1.6 ± 0.6
Polynuclear cells	3.8 ± 0.5	1.4 ± 0.6
Mononuclear cells	1.8 ± 0.9	1.2 ± 0.5
Edema	2.2 ± 0.5	1.4 ± 0.6
Epithelial hyperplasia	2.0 ± 0.7	1.0 ± 0.7

Supplementary Table S1. The histological scores

Samples were scored for the severity and character of the inflammatory response using a subjective grading scale.